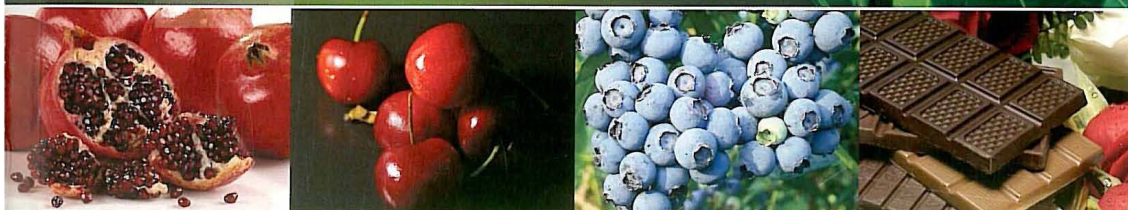




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Characterization of the phenolic constituents and the antioxidant activity of *Cytisus multiflorus*

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The specific interest in naturally occurring antioxidants has lead to an exponential investigation of many traditional medicinal plants, as they are potential candidates for finding new therapeutic and supplementary health products. *Cytisus multiflorus*, also known as White Spanish Broom, is a leguminous shrub native from Iberian Peninsula that is used in folk medicine mainly due to its diuretic and anti-inflammatory properties. Still, despite its common usage for centuries, this plant has been far less studied than other species of the same genus. This study aimed to determine the phenolic composition and to evaluate the antioxidant capacity of *Cytisus multiflorus*. For that, an ethanolic extract was prepared and its total phenolics and flavone contents were determined. The antioxidant activity was accessed by measuring the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging potential. Also, the extract was fractionated by reversed-phase HPLC and the major phenolic compounds of each fraction were identified by ESI-MS and MSⁿ analysis, in order to establish the specific phenolic profile of the plant. The total amount of phenolic compounds and of flavones accounted for 140 mg/g and 47 mg/g of the ethanolic extract from *Cytisus multiflorus*, respectively. Also, it exhibited a high antioxidative capacity, with an EC₅₀ value of 29,7 µg/ml. Moreover, the interpretation of the fragmentation pathways under ESI-MS/MS of the collected HPLC fractions allowed concluding that the main phenolic compounds in the plant included chrysin and some glycoside derivatives of luteonine and quercetin. Thus, the overall results suggest that the phenolic extract of the *Cytisus multiflorus* can be claimed as a good source of natural antioxidants.

CHARACTERIZATION OF THE PHENOLIC CONSTITUENTS AND THE ANTIOXIDANT ACTIVITY OF *CYTISUS MULTIFLORUS*



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INTRODUCTION

Cytisus multiflorus, also known as White Spanish Broom, is a leguminous shrub native from Iberian Peninsula that is used in folk medicine mainly due to its diuretic, hypoglycemic and anti-inflammatory properties. Still, despite its common usage for centuries, this plant has been far less studied than other species of the same genus. In this context, the present study aims to contribute for the chemical characterization of the *Cytisus multiflorus* specie by determining its phenolic composition and evaluating its antioxidant capacity.

METHODS

The total phenolic compounds and the flavone/flavonol contents of the ethanolic extract were determined by an adaptation of the Folin-Ciocalteu procedure [1] and by following the procedure of Popova *et al* [2], respectively. The phenolic characterization was performed by fractionation of the extract by reversed-phase HPLC and analysis of the major phenolic compounds by ESI-MS and MSn. The HPLC analysis was performed on a RP-C18 column 250 mm x 4 mm id, 5 µm bead diameter (Temperature of 30°C, flow rate of 1 mL/min). The mobile phase comprised (A) 0,1% formic acid in water and (B) 0,1% formic acid in acetonitrile and the solvent gradient started with 90% A and 10% B, reaching 40% B at 30 min, 5% B at 40 min, then returning to the initial conditions at 50 min. The antioxidant activity was accessed by measuring the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging potential [3].



RESULTS AND DISCUSSION

Table 1- Extraction yields, phenolic content and antioxidant capacity of *Cytisus multiflorus*

Mass (%)	^a Total phenolic (mg GAE/g)	^b Flavones/ Flavonols (mg QE/g)	^c DPPH (EC ₅₀) (mg/ml)
22,96%	140,39±11,67	47,43±1,31	0,53±0,05

Values are means±S.D. of three replicate analyses

^a Data expressed as milligrams of gallic acid equivalents (GAE) per gram of extract; ^b Data expressed as milligrams of quercetin equivalents(QE) per gram of extract; ^c EC₅₀ – concentration for a 50 % inhibition

The total amount of phenolic compounds and of flavones/flavonols accounted for 140 mg/g and 47 mg/g of the ethanolic extract from *Cytisus multiflorus*, respectively. Also, it exhibited a high antioxidative capacity, with an EC₅₀ value of 0,53 mg/ml.

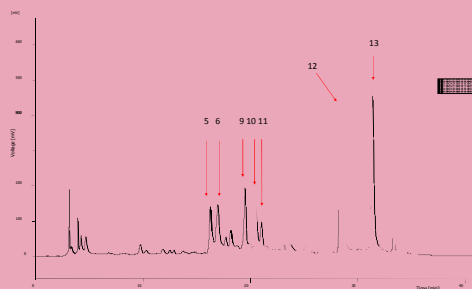


Fig 1- HPLC/UV profile at 280 of a (280nm) of the *Cytisus multiflorus* extract

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Phenolic characterization by fractionation of the *Cytisus multiflorus* extract by reversed-phase HPLC and analysis by ESI-MS and MSn.

Table 2- Chromatographic and Mass Spectral Characteristics of the phenolic constituents isolated from the *Cytisus multiflorus*

Peak	RT (min)	[M-H] ⁺ (m/z)	ESI ⁺ MS ⁿ	[M-H] ⁺ (m/z)	ESI ⁺ MS ⁿ	Compound
5	16,29	615	MS ² [615]: 579; MS ³ [579]: 459, 429	581	MS ² [581]: 449, 431, 329, 287	Luteolin-7-O-apiosyl- 8- C-glucoside derivative
		579	MS ² [579]: 459, 429, 357, 327, 285			
6	16,96	447	MS ² [447]: 357, 327, 285; MS ³ [357]: 339, 297, 285	449	MS ² [449]: 431, 413, 383, 329; MS ³ [431]: 413, 395, 353, 311; MS ⁴ [413]: 395; MS ⁵ [395]: 299, 287	Luteolin-8-C-glucoside
		579	MS ² [579]: 459, 429, 357, 327; - MS ³ [459]: 327; MS ⁴ [327]: 299; MS ⁵ [299]: 271, 255			
9	19,55	723	MS ² [723]: 579, 459, 357, 327; 725 MS ³ [579]: 459, 429, 357, 327; MS ⁴ [459]: 327; MS ⁵ [327]: 299	-	-	Kampferide derivative
		609	MS ² [609]: 343, 301; MS ³ [301]: 273, 257, 179, 151; MS ⁴ [179]: 151; MS ⁵ [151]: 107			
10	20,51	563	MS ² [447]: 285; MS ³ [285]: 241, 217, 199, 151	-	-	Luteolin-7-O-glucoside
		563	MS ² [563]: 517, 471, 413			
11	21,05	707	MS ² [707]: 645, 605, 563; MS ³ [563]: 443, 413; MS ⁴ [413]: 293; MS ⁵ [293]: 249, 175	-	-	Apigenin-6-C-Pentosyl-8-C-hexosyl Apigenin-6-C-Pentosyl-8-C-hexosyl derivative
		461	MS ² [461]: 415, 253; MS ³ [253]: 209			
12	28,44	451	MS ² [451]: 253; MS ³ [253]: 209	-	-	Chrysin glucoside derivative Chrysin glucoside derivative
		325	MS ² [325]: 183			
13	31,42	339	MS ² [339]: 183	503	MS ² [503]: 417, 255; MS ³ [417]: 255; MS ⁴ [255]: 213, 153	Metilgalate derivative Metilgalate derivative Chrysin-7-O-malonyl glucoside
		-	-			

CONCLUSION

The overall results suggest that the phenolic extract of the *Cytisus multiflorus* can be claimed as a good source of natural antioxidants. The extract is mainly composed of Chrysin glucoside derivatives (e.g. Chrysin- 7-O-malonyl glucoside) and/or Metilgalate derivatives. Several Luteoline glucoside derivatives and Quercetin-3-O-rutinoside are also present in this plant extract.